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Type II Collagenopathies: Are There Additional Family Members?

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The type II collagenopathies represent a group of chondrodysplasias sharing clinical and radiological manifestations which are expressed as a continuous spectrum of phenotypes, ranging from perinatally lethal to very mild conditions. Their common molecular bases are mutations in the type II collagen gene (COL2A1). We describe one case of lethal platyspondylic dysplasia, Torrance type, and a variant of lethal Kniest dysplasia, neither of which has been reported as a type II collagenopathy. Biochemical studies of cartilage collagens and morphological analysis of cartilage sections suggest that abnormalities of type II collagen structure and biosynthesis are the main pathogenetic factors in both cases. Thus, the phenotypic spectrum of type II collagenopathies might be greater than hitherto suspected.

KEY WORDS: chondrodysplasia; type II collagenopathies; platyspondylic dysplasia, Torrance type; Kniest dysplasia

INTRODUCTION

In spite of recent advances in the mapping and identification of genes responsible for bone dysplasias [Winter, 1995], the pathogenesis of the chondrodysplasias (CDXs) still remains unclear, mostly because of their striking clinical variability. However, one particular group of CDXs, comprising distinct clinical entities, has been ascribed to mutations in the COL2A1 gene encoding the α(1)II chain of the type II collagen. Defects in the structure or synthesis of this major constituent of the cartilage matrix and vitreous body appear to account for several phenotypes [reviewed in Spranger et al., 1994]. These so-called type II collagenopathies [Spranger et al., 1994] show an association of spinal, epi-, metaphyseal, and ocular abnormalities, with a clinical spectrum from perinatally lethal to mild conditions. They include achondrogenesis type II, hypochondrogenesis, spondyloepiphyseal dysplasia congenita (SEDc), Kniest dysplasia, Stickler arthroophthalmopathy, and autosomal-dominant spondylarthropathy. Recently, Tiller et al. [1995] have shown that spondyloepimetaphyseal dysplasia, Strudwick type, also is due to mutations in the COL2A1 gene. Abnormalities in type II collagen biosynthesis may also play a role in the pathogenesis of Goldblatt syndrome [Bonaventure et al., 1992].

The nature of the mutations and their localizations in the protein seem to explain the phenotypic differences, at least to a certain extent. Point mutations which introduce disturbance in the folding of the triple helix and mutations near the carboxy-terminal end of the molecule are associated with the most severe phenotypes, such as achondrogenesis type II [e.g., Chan et al., 1995] or hypochondrogenesis [e.g., Horton et al., 1992]. In contrast, premature stop codons leading to reduced amounts of normal type II collagen were found to produce Stickler syndrome, a phenotype of moderate severity [e.g., Ahmad et al., 1991, 1993].

We report on 2 further cases of perinatally lethal chondrodysplasias, one lethal platyspondylic chondrodysplasia, Torrance type, and one case similar to the lethal Kniest syndrome originally described by Chen et al. [1980] and further classified by Spranger and Maroteaux [1990].

In both conditions, biochemical and morphological investigations strongly indicate that defects in type II collagen biosynthesis might be the cause of the disease.

MATERIALS AND METHODS

Tissue Sources

Epiphyseal cartilage was removed from the femur several hours after death, and frozen at −20°C. Specimens were fixed in 4% buffered formaldehyde, and in 2.5% buffered glutaraldehyde (patient 2). For control studies,

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Dedicated to Jürgen W. Spranger on the occasion of his 65th birthday with admiration and best wishes.

cartilage was obtained from age-matched controls without any signs of connective tissue or growth disorders.

Protein Analysis

Extraction of collagen from cartilage, cyanogen bromide (CB) digestion, and electrophoretic analysis of collagen chains and CB peptides were performed as described previously [Freisinger et al., 1994a,b]. Total collagen content in cartilage hydrolysates was quantified by colorimetric determination of hydroxyproline amounts [Stegeman, 1958].

Morphological Examinations

Light microscopy and immunohistochemical staining were performed according to routine protocols following paraffin embedding. For immunostaining of type II collagen, a monospecific antibody was used (generously provided by K. von der Mark, Max Planck Institute, Erlangen). Appropriate tissue sections were therefore pretreated enzymatically, as described previously [Nerlich et al., 1993]. Transmission electron microscopy was performed according to standard procedures, with modifications as reported previously [Stoess, 1990].

RESULTS

Patient 1

This male fetus was the second child of healthy, unrelated parents with an unremarkable family history. Prenatal ultrasound examination at 24 weeks of gestation documented a male fetus with severe short-limb dwarfism. Clinical examination of the fetus after termination showed very short limbs, narrow thorax, and protruding abdomen. Radiological examination documented very short and broad tubular bones, with ragged metaphyseal borders and a "flaming" appear-

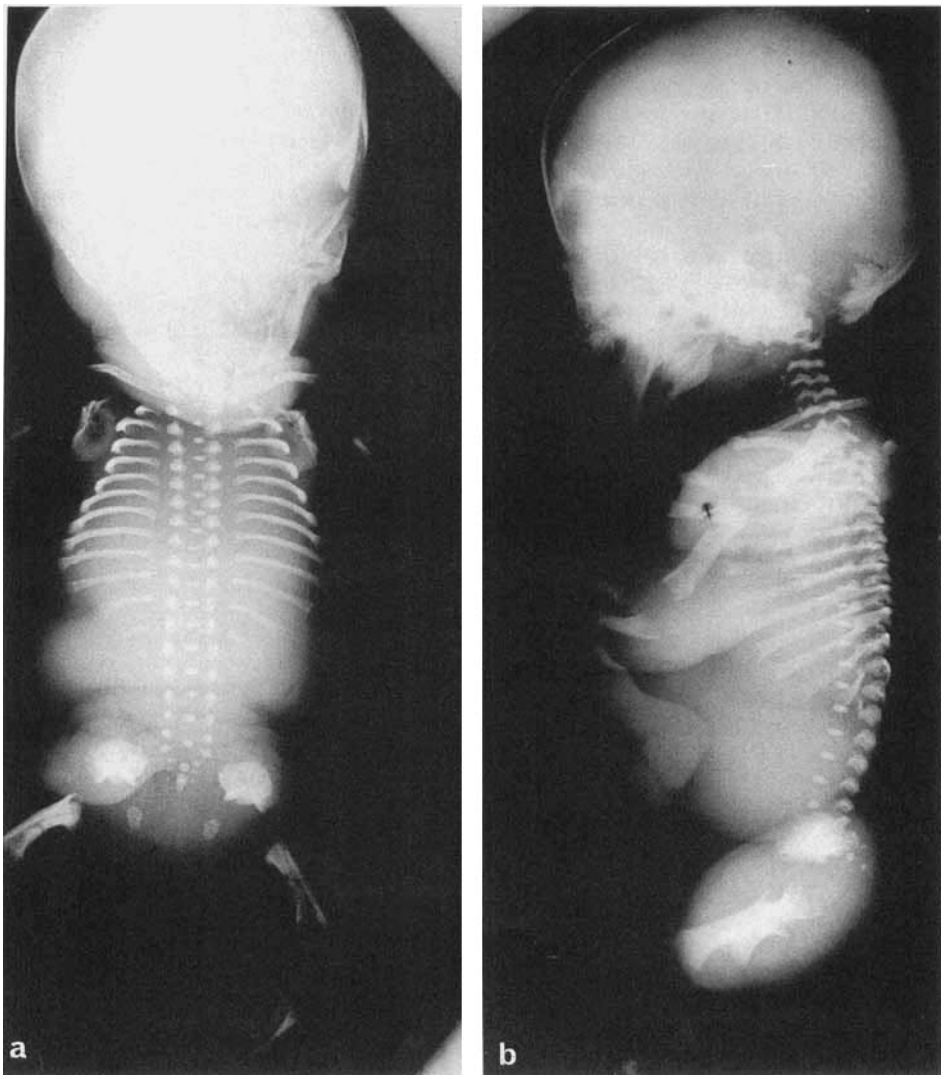

Fig. 1. Radiographs of patient 1 at 24 weeks of gestation. Note wafer-thin vertebral bodies **(a,b)**, and short and broad iliac bones with a concave inferior margin and a trident shape **(a)**. The tubular bones are short and broad with ragged metaphyseal borders. **b**: The ribs are short and their lateral ends are splayed.

ance of the femoral metaphyses, wafer-thin vertebral bodies, and short and broad ilia with irregular, slightly concave inferior margins and trident conformation (Fig. 1a,b). These radiological signs were consistent with the diagnosis of lethal platyspondylic chondrodysplasia. The histomorphological aspect of the epiphyseal cartilage was evocative of the Torrance subtype of platyspondylic dysplasia (V. Stanescu, personal communication).

Protein Analysis

Determination of total collagen content in cartilage showed a reduction by approximately 20%, when compared to age-matched controls. SDS-polyacrylamide-electrophoresis (SDS-PAGE) of cartilage collagen showed an α1(II) chain with normal mobility, as well as a slower-migrating band with lower intensity. The abnormal chain coprecipitated with the α1(II) chain on selective precipitation with 0.86 M NaCl (Fig. 3a), suggesting that it represented an overmodified α1(II) chain rather than the α3(XI) chain of type XI collagen. The amount of abnormally migrating chains was higher in the neutral-salt soluble fraction than in the pepsin-soluble fraction. Selective precipitation of type XI collagen with 1.2 M NaCl, and type IX collagen with 2.0 M NaCl, showed that both types were present in normal quantities and migrated normally (not shown). There was a higher content of type I collagen chains as compared to control.

Electrophoresis of the CB-digested collagen peptides demonstrated normal and delayed migrations of α1(II)CB 10.5, CB 8, CB 11, and CB 12, while CB 9.7 was unaffected (Fig. 4). This result further emphasized the hypothesis that the slower-migrating band corresponded to an overmodified α1(II) chain.

Patient 2

The girl was born at term as the third child of healthy, nonconsanguineous parents with an unremarkable family history. Clinical examination showed disproportionate dwarfism (birth weight, 2,400 g; length, 39 cm; head circumference, 33 cm), markedly short limbs, a bell-shaped chest, protruding abdomen, and hypoplastic midface. After resuscitation for respiratory distress she required mechanical ventilation until she died at age 6 months due to severe pneumonia. During this period her length increased by only 1 cm.

Radiological examination at different ages showed flat and broad vertebrae with slightly irregular endplates and coronal clefting (Fig. 2a,c). The ribs were short, with anterior flare. The iliac wings were small, the basilar portion of the ilia was hypoplastic, the ischial bones were short and broad, and the pubic bones were not ossified (Fig. 2b). The tubular bones were markedly short, especially in the proximal segments, with wide, irregular, convex metaphyses and relatively broad diaphyses (Fig. 2d,e). Ossification was delayed. There were no gross abnormalities of the inner organs, eyes, and palate.

Protein Analysis

Total collagen content in cartilage was not reduced as compared to controls. SDS-PAGE of pepsin soluble car-

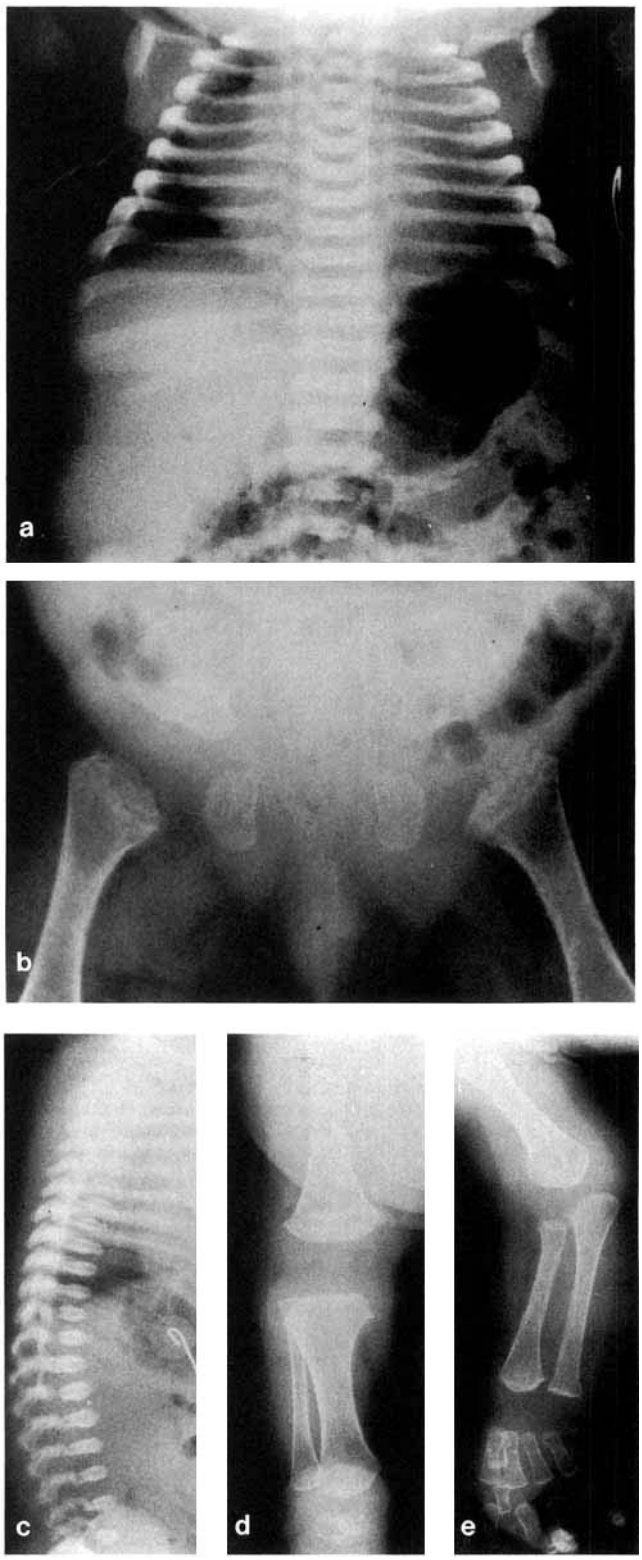

Fig. 2. Radiographs of patient 2 at age 3 months, showing short ribs, especially in the upper half of the thorax (**a**), platyspondyly, and coronal clefting (**a, c**). The basilar portion of the iliac bones is hypoplastic, and the ischial bones are short and broad. **b:** There is no ossification of the pubic bones. The tubular bones are short. **b, d, e:** The metaphyses are large and convex with an irregular border, especially of the upper femoral metaphyses. **d:** The diaphyses appear relatively broad.

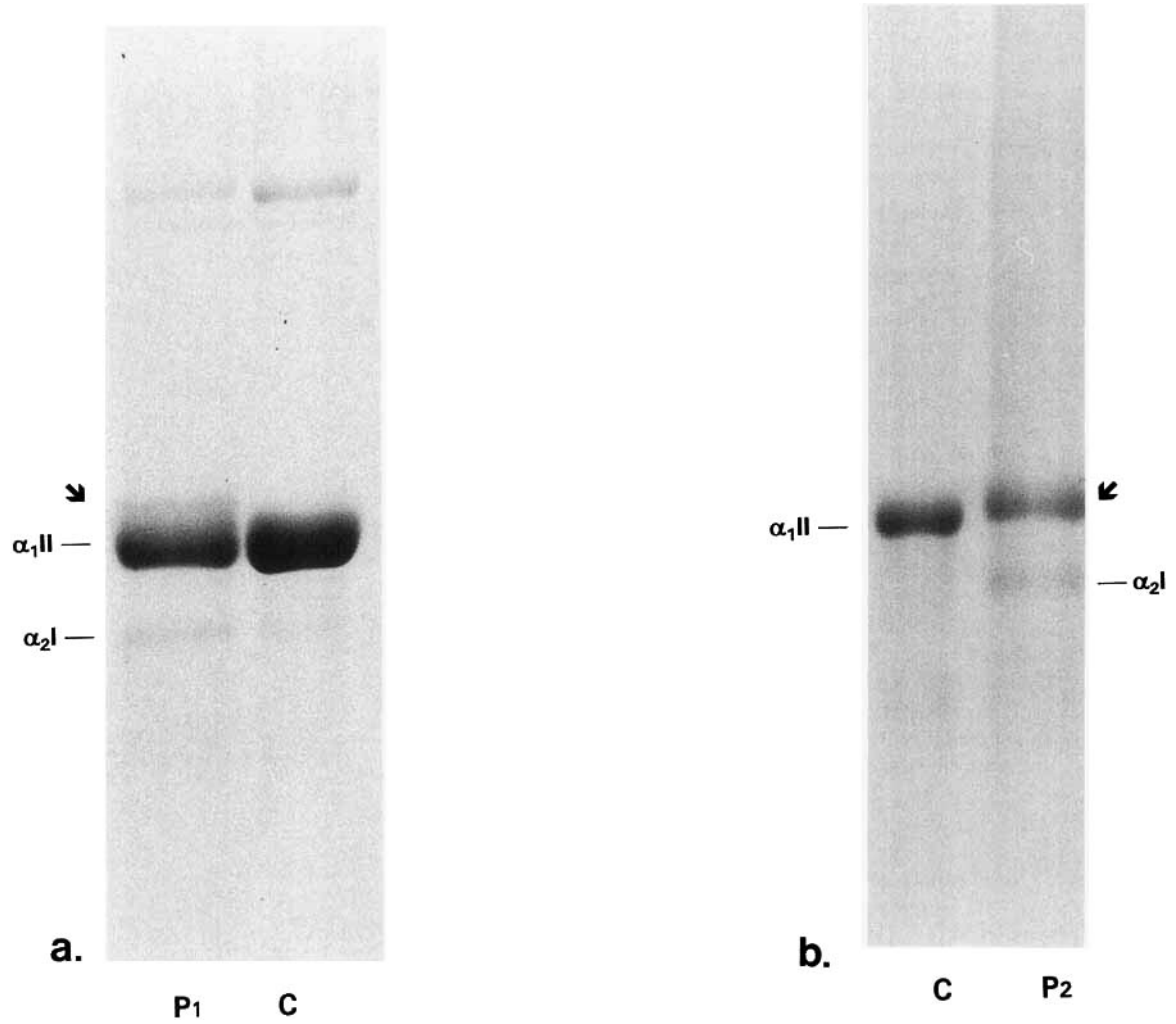

Fig. 3. Electrophoresis in SDS 5%-polyacrylamide of pepsin-solubilized collagen from cartilage after selective precipitation of the α(1)II chains with 0.86 M NaCl (stained with Coomassie blue). C, control; P1, patient 1; P2, patient 2. **a:** Note more slowly-migrating α(1)II chain (↘) above the normally-migrating α(1)II chain and the presence of type I collagen (α(2)I) in patient 1. **b:** In patient 2, only abnormally migrating α(1)II chains (↙) and some type I collagen was detected.

tilage collagen showed a retarded migration of the α1(II) chains. No normally-migrating α1(II) chains were detectable (Fig. 3b). Type IX collagen and type XI collagen were present in normal quantities and migrated normally. Type I collagen chains were detected in higher amounts than in controls. SDS-PAGE of CB-digested collagen peptides showed slowly-migrating α1(II) CB 10.5, CD 8, CB 11, and CB 12, while peptide CB 9.7 was unaffected. As in the first patient, this result indicated that the band with delayed mobility corresponded to overmodified α1(II) chains.

Histology

The resting cartilage showed focally-enhanced cellularity with lacunar chondrocytes. The number of vascular channels appeared significantly increased, with enhanced vascular fibrosis. In the growth plate the proliferative and hypertrophic zones were markedly reduced and irregularly formed (Fig. 5a). The transition zone between resting and proliferating chondrocytes showed a remarkable vacuolization of the cells (Fig. 5b). A typical "Swiss cheese" pattern was not seen. Immunolocalization study of type II collagen demonstrated a homogenous expression of this collagen throughout the cartilage.

Ultrastructural Data

Transmission electron microscopy of the patient's cartilage showed chondrocytes with dilated cisternae of the rough endoplasmic reticulum (RER) of variable size containing a granular material (Fig. 6a). The collagen fibrils were thin. An alteration of areas presenting normally packed fibrils, with areas exhibiting a significantly reduced fibril density, was observed (Fig. 6b).

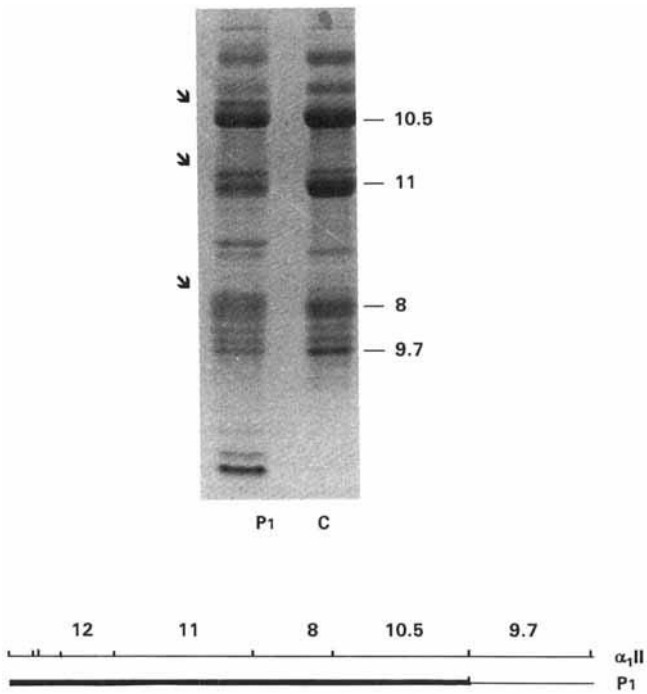

Fig. 4. Electrophoresis in SDS 10–17% polyacrylamide of CB peptides of pepsin-solubilized cartilage collagen after selective precipitation of α(1)II chains with 0.86 M NaCl (stained with Coomassie blue). C, control; P1, patient 1. Arrows indicate more slowly-migrating CB peptides of type II collagen in the patient's sample. CB peptide map of the α(1)II-procollagen molecule is shown below. Underline indicates overmodified portion of the molecule.

DISCUSSION

The term "type II collagenopathies" was introduced by Spranger et al. [1994] to describe a group of distinct CDXs sharing some clinical and radiographic manifestations representing the phenotypic expression of mutations in the COL2A1 gene. Here we describe 2 patients with two different forms of CDX, which up to now were not included in the phenotypic spectrum of type II collagenopathies. In both cases, biochemical screening of cartilage collagen abnormalities documented alterations of type II collagen electrophoretic mobility.

In the first patient, on the basis of radiologic and histologic examination, platyspondylic dysplasia, Torrance type, could be diagnosed [Horton et al., 1979; Spranger and Maroteaux, 1990]. The cause of this extremely rare disorder is still unclear, and to our knowledge no pathogenetic studies have been performed.

Our studies show the presence of overmodified type II collagen in cartilage. The higher content of abnormal collagen in the salt-soluble fraction suggests that the abnormal α1(II) chains are not appropriately integrated in stable crosslinked fibrils and could be partially degraded, thus explaining the reduced collagen content found in cartilage. The presence of significant amounts of type I collagen in this case might be related to previous observations of variable quantities of type I collagen in the most severe forms of type II collagenopathies, including achondrogenesis type II [Eyre, 1988; Chan et al., 1995] and hypochondrogenesis [Freisinger et al., 1994b]. It may eventually be explained by production of type I collagen to compensate for defective type II collagen synthesis.

CB-peptide analysis indicated overmodification of the triple helix initiated within the domain defined by peptide CB 10.5. Consistent with previous reports, posttranslational overmodifications were attributed to glycine substitution in the type II collagen gene (COL2A1). Unfortunately, SSCP analysis of the whole exon encoding the triple helix has failed to detect such a mutation. Therefore, whether overmodification of the α(1)II chain is directly related to the phenotype or only represents a secondary consequence of another primary defect remains to be elucidated.

The second patient presented a less severe phenotype. The radiological examination showed several signs compatible with the diagnosis of lethal Kniest syndrome [Spranger and Maroteaux, 1990]. However, the lack of some clinical characteristics such as cleft palate, pectus carinatum, eye defects, and clubfeet indicate that this patient could represent a Kniest-like dysplasia. This assertion is supported by the peculiar histologic aspect of the cartilage, which did not show the typical perilacunar foamy appearance seen in other individuals with Kniest dysplasia [Horton and Rimoin, 1979]. As in the first patient, we were able to demonstrate the presence of overmodified type II collagen. In contrast, no significant amounts of normal type II collagen chains were detectable in this case. Total collagen content was not reduced. This suggested that the abnormal collagen chains were incorporated into the cartilage matrix. This was supported by the immunohistochemical findings of rather normal type II collagen distribution within the cartilage. The ultrastructural examination, showing thin collagen fibrils which were only focally reduced in density, also favored this hypothesis. The dilated RER of the chondrocytes is likely to reflect delayed secretion of the altered type II collagen chains. The presence of a relatively well-conserved cartilage matrix could account for the lower phenotypic severity than in the first patient. Identification of the mutation has not yet been achieved in this patient, and is still under investigation.

The biochemical findings in both patients are comparable to those found in other type II collagenopathies [reviewed in Spranger et al., 1994]. In milder forms of Kniest dysplasia, several mutations of the COL2A1 gene and their biochemical consequences have been found [Bogaert et al., 1994; Wilkin et al., 1994]. Although the diagnosis of lethal Kniest dysplasia is not definitively established in our second patient, a type II collagen defect appears the most likely explanation for the phenotype. The first case, in contrast, should be regarded as a disorder clinically distinct from the known lethal type II collagenopathies.

Even if direct proof for mutation in the COL2A1 gene of these 2 patients is still lacking, we think that they may represent additional members of the type II collagenopathies "family." It remains unclear whether this

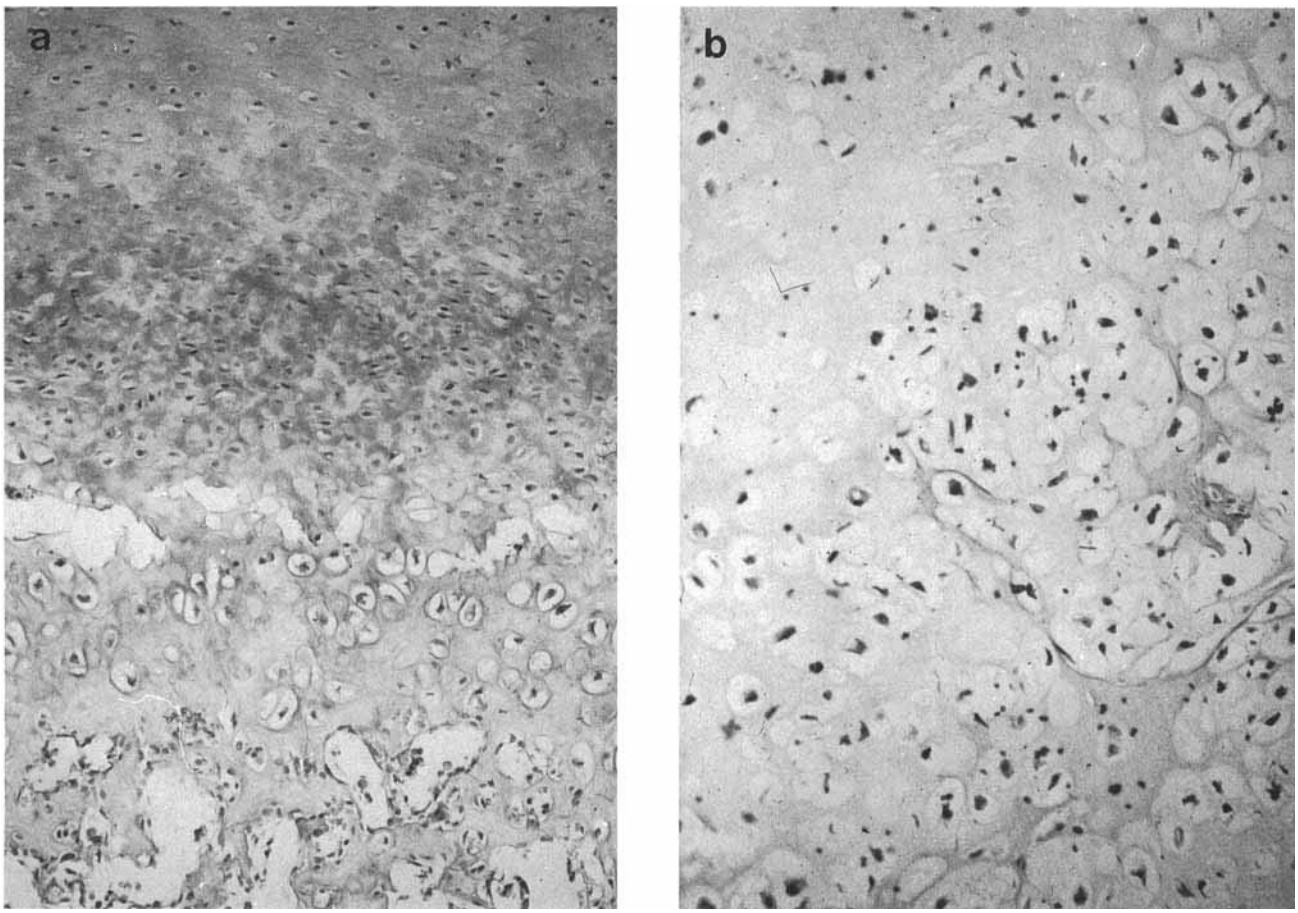

Fig. 5. Histological aspects of osteochondral transition zone in patient 2 (Alcian blue staining, ×400). **a:** Note marked reduction of proliferative and hypertrophic zones in the growth plate, and the disorganized columns. **b:** Strong vacuolization of cells in the transition zone between resting and proliferating chondrocytes.

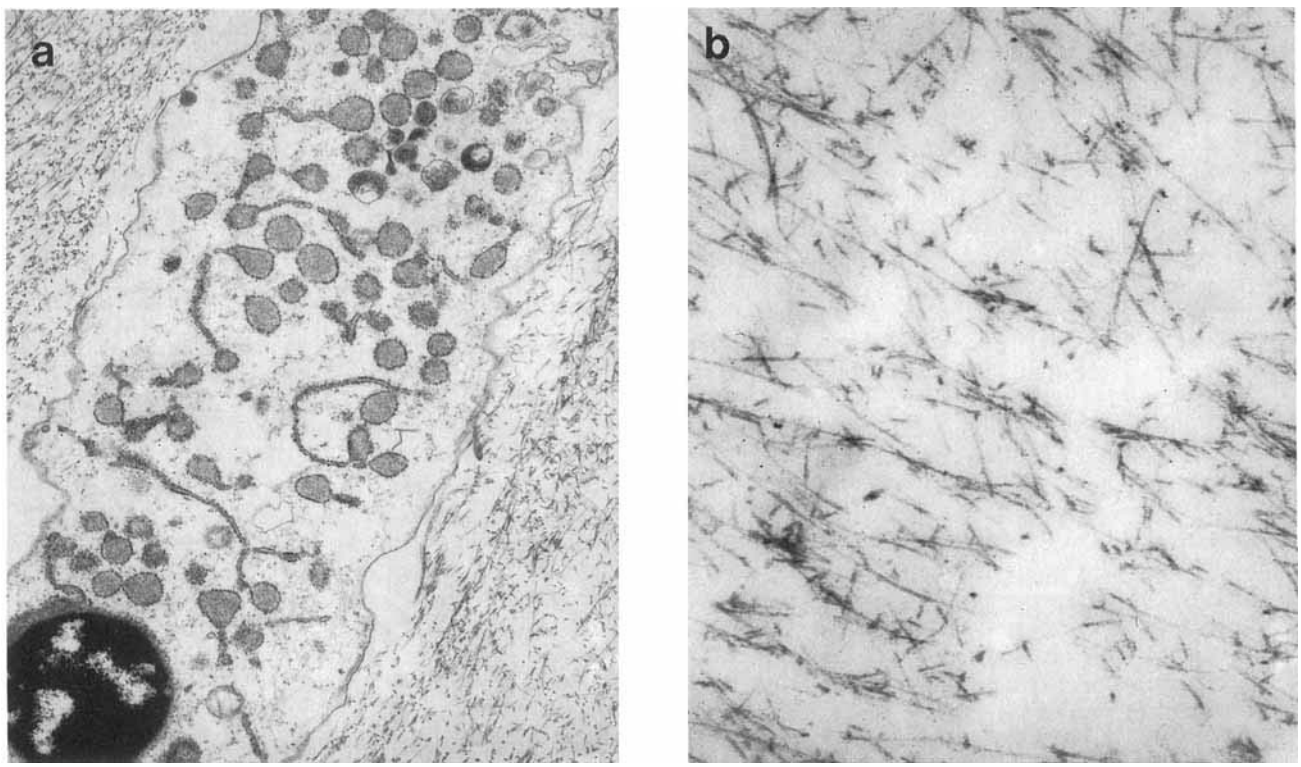

Fig. 6. Electron micrographs of cartilage (patient 2). **a:** Cisternae of RER are dilated (×7,000). **b:** Collagen fibrils are thin and loosely packed (×20,000).

phenotypic variation is only related to the position and/or the nature of the COL2A1 mutation, or whether further modulating factors are involved in their pathogenesis.

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